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Bukti tanggal 10 Oktober 2017

**Recd: 10/10/17**

**ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA SECRETION OF CHRONIC SUPPURATIVE OTITIS MEDIA PATIENTS**

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**ABSTRACT**

The aims of this research were to isolate and identify the pathogenic bacteria in the secretion of Chronic Suppurative Otitis Media (CSOM) patients as the development of Lactic Acid Bacteria (LAB) analysis in Virgin Coconut Oil (VCO) fermentation process. It is expected that LAB in the VCO could be antimicrobial/antibacterial of bacteria in the secretion of CSOM patients. This research was conducted in 2 stages; (1) isolate the bacteria in the secretion of CSOM patients using blood agar and dilution method; (2) identify the isolates morphologically, physiology, and other biochemical test. There are 126 isolates and 5 kinds of pathogenic bacteria (*Pseudomonas aureginosa*, *Staphilococcus aureus*, *Staphilococcus epidermidis*, *Proteus mirabilis*, *Klebsiella Sp*) and one kind of fungi (*Candida sp*) as the result. The samples of CSOM patients are 60% above aged 20 and 40% below it, and equal balance of percentage between male and female.

**Keywords:** Pathogenic bacteria isolation, Secretion of CSOM patients, Chronic Suppurative Otitis Media, Virgin Coconut Oil (VCO), Lactic Acid Bacteria (LAB) .

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**INTRODUCTION**

Chronic Suppurative Otitis Media (CSOM) is a kind of ear disease that commonly suffered by children and causes deafness, even death<sup>1, 2</sup>. It usually attacks people in developing countries such as India, Nepal, Vietnam and also Indonesia<sup>3, 4</sup>. Indonesian calls it 'congek', and is one of deadly diseases because there is tympanic membrane perforation and secretion that flows from the outer ear continuously or temporary which can cause dangerous complication such as brain abscess and meningitis<sup>5, 6, 7</sup>. CSOM derives from the late effect of treatment for acute otitis media patient, or poor hygiene practice, high virulence, and weak immune system due to malnutrition<sup>8</sup>.

Some researchers have tried to isolate the pathogenic bacteria in the secretion of CSOM patients, and one of them was an Indian researcher<sup>4</sup>. He said that from 80 samples of CSOM patients, there were few pathogenic bacteria; *Staphilococcus aureus*, *Pseudomonas sp*, *Escherichia coli*, and *Klebsiella sp*. Apparently, 18% of the bacteria were resistance toward antibiotic like methicillin, and sensitive toward amikacin, chloramfenicol and piperacillin.

The most pathogenic bacteria found in CSOM that highlighted CSOM object were *Streptococcus Pnemonea* and a virus<sup>1</sup>. those pathogenic bacteria previously mentioned were

aerobic and anaerobic. *P.aeruginosa*, *S.aureus*, *S. pyogenes*, *K.pneumoniae*, *H.influenzae*, *Bacteroides* and *Proteus sp* were mostly found along with the mixture of aerobic and anaerobic bacteria that form a layer called biofilm<sup>9</sup>.

Meanwhile<sup>10, 11</sup> that there were bacteriocins in Lactic Acid Bacteria (LAB). Bacteriocins can kill pathogenic bacteria but it is not dangerous for non-pathogenic bacteria<sup>12</sup>. Antibacterial test and anti-fungal test using 5 samples of bacteria (*E.coli* NBRC14237, *Staphylococcus aureus* NBRC 13276, *Bacillus subtilis* BTCCB, *Salmonella thypii*, and *Listeria monocytogenes*) and 2 samples of fungi (*Aspergillus niger* and *Candida sp*) in VCO fermentation process, recently found also spices that have the ability as antimicrobial<sup>13</sup>, there were pathogenic bacteria of CSOM patients found among samples of bacteria (*S. Aureus*). There was a fungus of CSOM patient found between the samples<sup>14</sup>. Because oil layer in VCO is contained LAB that can inhibit the growth of pathogenic bacteria, thus it is hoped that pathogenic bacteria in secretion of CSOM patients can be inhibited as well by the LAB

## EXPERIMENTAL

### Material and Methods

#### Materials

Material for this study was ear liquid of 126 CSOM patients in X Hospital. The media to grow the bacteria during conventional isolation and identification processes were blood agar and McConkey agar. More over the other materials taken were MRS (15g peptone, 5g yeast extract, 10g dextrose, 5g tomato juice, 2g monopotassium phosphate, and 1g polysorbate 80), Luria-Bertani medium (10g tryptone, 5g yeast extract, and 10g NaCl), sodium acetate, liquid nitrogen, methylene blue, sterile aquadest, sodium azide, HCl 6 N, ampicilin, ammonium sulfate, Tris-HCl 50 mM pH 7.4, NaCl 1 M, Tris-HCl 100nM pH 8.5, glycerol, isopropanol, 70% ethanol, ammonium molybdate, trisodium citrate, aquabidest, methanol, pure Agar, 70% alcohol, 96% ammonium sulfate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, Aquadest, buffer solution pH 7, technical hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), potassium hydroxide (KOH), phenolphthalein (PP) analysis, technical starch, and lactose broth.

#### Methods

There were 2 stages conducted in this study; (1) Isolation of pathogenic bacteria of 126 CSOM patients; (2) Identification of pathogenic bacteria using gram-negative and positive test, bacterial staining test, morphology test, and biochemical test such as catalase test and other carbohydrate tests.

#### General procedure

*The Isolation of Pathogenic Bacteria in the Secretion of the Patients.* The isolation stage was done before doing the identification of pathogenic bacteria in the secretion of 126 CSOM patients. Pathogenic bacteria from 126 CSOM patients were isolated by using dilution method up to 10<sup>-7</sup> dilution level, whereas the media used to isolate these bacteria were blood agar and McConkey agar. Streak the bacteria for a single colony so it could become the isolate of the pathogenic bacteria. At the same time when the secretion was scratched in blood agar, it was also enriched in tiogikolat. The sample that has been enriched and planted in blood agar media would

be taken when there was no bacterium grew in the media. Usually in each CSOM patient there was one isolate produced.

*The Identification of Isolate of Pathogenic Bacteria.* Then the isolates which have been collected were morphologically identified referred to their colony pattern and color. Besides that, positive and negative-gram tests, biochemical test such as catalase test, starch test, and novobiocin test were performed as well.

## RESULTS AND DISCUSSION

Data of secretion taken from CSOM patients in this study could be seen in Table 1 below:

Table 1. Sample Distribution

No.	Patient	Amount	%
1.	Children (under 13 year-old)	51	40
2.	Adult	75	60
3.	Male	72	57
4.	Female	54	43

The table above showed that 57% of the data were taken from male. The patients chosen were 40% children and 60% adult. Yaor, MA in his previous research said that CSOM can attack children and adult<sup>15</sup>. He confirmed that of 73 studied CSOM patients aged 9 to 84 year-old, 17 of them (24%) were children aged 9-15. The 40% number occurred to children were because of poor hygiene practice therefore was easily infected by the bacteria and another side Shyamala found that 70% of CSOM patients were children aged 0-20. It was similar with Moris statement that mostly those who suffer CSOM were children.<sup>16, 4, 3, 17</sup>

### *The Isolation of Pathogenic Bacteria.*

This study found one kind of pathogenic bacteria of the CSOM patient in the isolation process, so there were 126 isolates gathered at the end. Compared to Suryani, and R. Shyamala<sup>16, 18</sup> study, he stated that each patient had one isolate; 64% of 192 samples, while 34% of them had more than one, and 5.33% of the isolated secretion produced fungi.

### *Morphologic Identification of Pathogenic Bacteria.*

The result of pathogenic bacteria identification of 126 secretions of CSOM patients can be seen in Table 2 below:

Table 2. Morphologic Analysis of the Isolates

No.	Macroscopic Characteristics of Isolate	Isolate	Number of Isolate
1.	<ul style="list-style-type: none"> <li>• The color is grayish white</li> <li>• The shape like a fragment</li> <li>• The size is 6-15 mm</li> <li>• The texture is rough</li> <li>• Greenish pigment</li> <li>• Smelly</li> <li>• Gram-negative (bacilli)</li> </ul>	<i>Pseudomonas aureginosa</i>	74 (58,7%)
2.	<ul style="list-style-type: none"> <li>• Circular shape</li> <li>• The size is medium</li> <li>• Convex</li> <li>• Possessing flagella</li> <li>• Spread</li> <li>• Smell salty</li> <li>• Gram-negative (bacilli)</li> </ul>	<i>Proteus mirabilis</i>	21 (16,6%)
3.	<ul style="list-style-type: none"> <li>• Circular shape</li> <li>• The size is big</li> <li>• Convex</li> <li>• Mucoid</li> <li>• Shiny</li> <li>• The edge is smooth</li> <li>• Gram-negative bacilli</li> </ul>	<i>Klebsiella</i>	7 (5%)
4.	<ul style="list-style-type: none"> <li>• Circular shape</li> <li>• Slightly Convex</li> <li>• The edge is smooth</li> <li>• The color is yellowish white</li> <li>• The size is 2-5 mm</li> <li>• <math>\beta</math> hemolytic</li> <li>• Positive-gram (cocci)</li> <li>• Aciniform (Grouped like grapes)</li> </ul>	<i>Staphylococcus aureus</i>	14 (11%)
5.	<ul style="list-style-type: none"> <li>• Circular shape</li> <li>• Slightly Convex</li> <li>• The edge is smooth</li> <li>• The color is white</li> <li>• The size is small</li> <li>• Cocci</li> <li>• Positive-gram</li> <li>• Aciniform (Grouped like grapes)</li> </ul>	<i>Staphylococcus epidermidis</i>	4 (3%)

Meanwhile in fungi identification, when observation of isolation process conducted, there was a colony found with the presence of hypha in it. Then, continued by gram staining test and obtained positive pseudohyphae as the result. Then, the samples were grown in blood agar and Saboraud agar. Resulted that the colony grew in Saboraud agar instead of blood agar, in the form of circular shape, white, and slightly mucoid. The result can be seen in Table 3 below:

**Table 3. Morphologic Analysis of Fungi from the isolates of Pathogenic Bacteria**

No.	Characteristics	Isolate	No of Isolate
1.	<ul style="list-style-type: none"> <li>•Positive-gram</li> <li>•Pseudohypha +</li> <li>•Didn't grow in blood agar</li> <li>•Grow in saboraaud</li> <li>•Circular shape, white, and slightly mucoid</li> </ul>	<i>Candida sp</i>	6 (4,7%)

*Biochemical Test.* The result of biochemical test of the isolates can be seen in Table 4 below:

**Table 4. Result of Biochemical Test of the Isolate**

No.	Test	Result	No of Isolate	Isolate
1.	TSIA	K/K	74	<i>Pseudomonas aureginosa</i>
2.	Gas	+		
3.	H <sub>2</sub> S	-		
4.	SC	+		
5.	Sulfur	+		
6.	Indole	-		
7.	Motile	+		
1.	TSIA	K/A	21	<i>Proteus mirabilis</i>
2.	Gas	+		
3.	H <sub>2</sub> S	+		
4.	SC	+		

5.	Sulfur	+		
6.	Indole	-		
7.	Motile	+		
1.	TSIA	A/A		<i>Klebsiella</i>
2.	Gas	+		
3.	H <sub>2</sub> S	-		
4.	SC	+		
5.	Sulfur	-		
6.	Indole	-		
7.	Motile	-		
1.	Catalase	+	14	<i>Staphylococcus aureus</i>
2.	Gas	+		
3.	Coagulase	+		
4.	Novobiocin	Sensitive		
1.	Catalase	+	4	<i>Staphylococcus epidermidis</i>
2.	Gas	+		
3.	Coagulase	-		
4.	Novobiocin	Sensitive		

From morphology identification result mentioned in Table 2 and 3, it can be seen the shape, color, size of colony from each isolate and also the gram test result. The above result showed the types of pathogenic bacteria in the secretion of CSOM patients in X Hospital, they were *Pseudomonas aureginosa* (58,7%), *Staphylococcus aureus* (11 %), *Staphylococcus epidermidis* (3%), *Proteus mirabilis* (16,6 %), *Klebsiella sp* (5%) and 1 fungi *Candida sp* (4,7%). This result is supported by the result of biochemical test on each isolate such as Catalase,



**Koagulase**, formed gas, and Novobiocin test as stated in table 4. Nevertheless the result was in the same agreement with other experts, there was a few differences on the pathogenic bacteria and fungi found in the secretion of CSOM patients. Sthrestha et.al (2011) said that pathogenic bacteria and pathogenic fungus of CSOM patients were *Staphylococcus aureus* 32,2%, *Streptococcus pnemoni* 6,1 %, *Pseudomonas aureginosa* 26,9 % , *Klebsiella sp* 10,4 %, *Proteus mirabilis* 6,9 % , *E.coli* 6,9%, fungi *Aspergillus sp* 6,9 % *Candida sp* 2,6 %.

### CONCLUSION

From the result mentioned above, it can be concluded that there were 126 isolates of pathogenic bacteria from the secretion of 96 CSOM patients. More over, there were 5 kinds of pathogenic bacteria found in the secretion of CSOM patients in X Hospital; *Pseudomonas aeruginosa*; *Klebsiella*, *Proteus*; *Staphylococcus aureus*; *Staphilococus epidermidis* and one species of fungi *Candida spp*.

### ACKNOWLEDGEMENT

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4. The Head of Basic Laboratory of Kopertis X that also gave us permission and help to finish this research.

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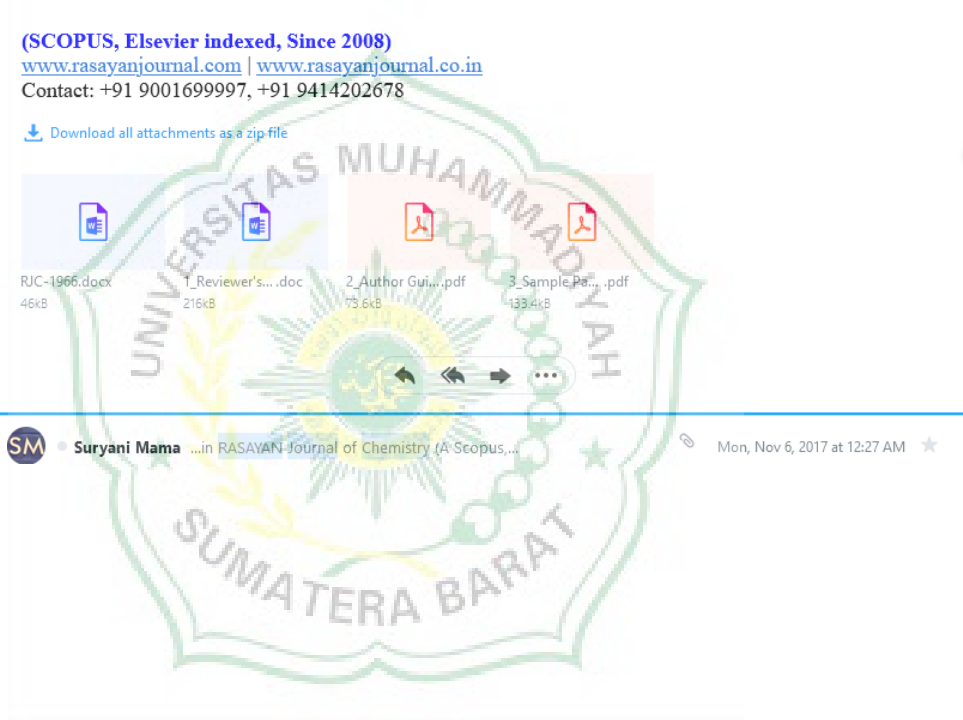
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## Reviewer's Report

### SECTION I: Details of Manuscript

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Manuscript Number:	RJC-1966
Title:	<b>ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA SECRETION OF CHRONIC SUPPURATIVE OTITIS MEDIA PATIENTS</b>
Authors:	Suryani Suryani; Zulmardi; Abdi Dharma; Nasril Nasir
Date of receiving by Reviewer:	
Date of submission From Reviewer:	

### SECTION II: Comments per Section of Manuscript

General comment:	<p>Overall, this research has implemented the right and good methods in order to get rich data for its result. The methods which are conducted here are Isolation of pathogenic bacteria of 126 CSOM patients, and Identification of pathogenic bacteria using gram-negative and positive test, as have been proposed by experts in their researches.</p>
Introduction:	<p>This introduction has fulfilled the requirement of what should be stated as the background of the research. Rich information of the topic of this research and provide it with experts statement as well that concerns on the similar field.</p>
Methodology:	<p>It has applied the appropriate methods which should be done within the research analyzing Pathogenic Bacteria on the secretion of chronic suppurative otitis media patients. It can be seen from the rich data obtained.</p>



Results:	The result gives depth analysis on the data perceived by providing it with particular table for particular focus of concern.
Discussion:	The discussion shows its clarity in presentation by elaborating the data within each table concisely and neatly.

## SECTION II (Cont.)

Bibliography/References:	The references taken as the guideline for conducting this research are appropriate and fit with the topic of discussion.
Others:	Perhaps, it needs a bit addition on the conclusion part. Develop the result paragraph by presenting more brief explanation about them in order to make the data becoming more credible.
Decision:	This article can be accepted as the one who put much concern on the field designated as it provides much contribution on the chronic suppurative otitis patients.

## SECTION III - Please rate the following: (1 = Excellent) (2 = Good) (3 = Fair) (4 = poor)

Originality:	1
Contribution to the Field:	1
Technical Quality:	2
Clarity of Presentation :	1
Depth of Research:	1

## SECTION IV - Recommendation: (Kindly Mark with an X)





Accept As Is:	
Requires Minor Revision:	<b>x</b>
Requires Moderate Revision:	
Requires Major Revision:	
Rejected for publication in RJC(Please give reason):	

## SECTION V: Additional Comments

Please add any additional comments (Including comments/suggestions regarding online supplementary materials, if any).





## Reviewer's Report

### SECTION I: Details of Manuscript

Reviewer's Name:	
E-Mail:	
Manuscript Number:	RJC-1966
Title:	ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA SECRETION OF CHRONIC SUPPURATIVE OTITIS MEDIA PATIENTS
Authors:	Suryani Suryani, Zulmardi, Abdi Dharma, and Nasril Nasir
Date of receiving by Reviewer:	2017-10-28
Date of submission From Reviewer:	2017-11-03

### SECTION II: Comments per Section of Manuscript

General comment:	The work is original and well written, somehow the use of proper English should be implemented in the revision version.
Introduction:	Good to explain the need of the work to be done.
Methodology:	The methodology is the standard one.
Results:	<ol style="list-style-type: none"><li>1. The results section is detailed and well presented.</li><li>2. The tables are needed to be organized to simplify them.</li></ol>
Discussion:	It is still needed to discuss the results further, compare them with existing data to explain the findings.



## SECTION II (Cont.)

Bibliography/References:	The references used in this article are the updated ones.
Others:	Aknowledgment section should be simplified.
Decision:	Accepted.

## SECTION III - Please rate the following: (1 = Excellent) (2 = Good) (3 = Fair) (4 = poor)

Originality:	2
Contribution to the Field:	2
Technical Quality:	2
Clarity of Presentation :	2
Depth of Research:	2

## SECTION IV - Recommendation: (Kindly Mark with an X)

Accept As Is:	
Requires Minor Revision:	
Requires Moderate Revision:	X
Requires Major Revision:	
Rejected for publication in RJC(Please give reason):	

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Please add any additional comments (Including comments/suggestions regarding online supplementary materials, if any).



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Date of receiving by Reviewer:	
Date of submission From Reviewer:	

### SECTION II: Comments per Section of Manuscript

General comment:	<p>Overall, this research has implemented the right and good methods in order to get rich data for its result. The methods which are conducted here are Isolation of pathogenic bacteria of 126 CSOM patients, and Identification of pathogenic bacteria using gram-negative and positive test, as have been proposed by experts in their researches.</p>
Introduction:	<p>This introduction has fulfilled the requirement of what should be stated as the background of the research. Rich information of the topic of this research and provide it with experts statement as well that concerns on the similar field.</p>
Methodology:	<p>It has applied the appropriate methods which should be done within the research analyzing Pathogenic Bacteria on the secretion of chronic suppurative otitis media patients. It can be seen from the rich data obtained.</p>



Results:	The result gives depth analysis on the data perceived by providing it with particular table for particular focus of concern.
Discussion:	The discussion shows its clarity in presentation by elaborating the data within each table concisely and neatly.

## SECTION II (Cont.)

Bibliography/References:	The references taken as the guideline for conducting this research are appropriate and fit with the topic of discussion.
Others:	Perhaps, it needs a bit addition on the conclusion part. Develop the result paragraph by presenting more brief explanation about them in order to make the data becoming more credible.
Decision:	This article can be accepted as the one who put much concern on the field designated as it provides much contribution on the chronic suppurative otitis patients.

## SECTION III - Please rate the following: (1 = Excellent) (2 = Good) (3 = Fair) (4 = poor)

Originality:	1
Contribution to the Field:	1
Technical Quality:	2
Clarity of Presentation :	1
Depth of Research:	1

## SECTION IV - Recommendation: (Kindly Mark with an X)





Accept As Is:	
Requires Minor Revision:	<b>x</b>
Requires Moderate Revision:	
Requires Major Revision:	
Rejected for publication in RJC(Please give reason):	

## SECTION V: Additional Comments

Please add any additional comments (Including comments/suggestions regarding online supplementary materials, if any).







## Reviewer's Report

### SECTION I: Details of Manuscript

Reviewer's Name:	
E-Mail:	
Manuscript Number:	RJC-1966
Title:	<b>ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA SECRETION OF CHRONIC SUPPURATIVE OTITIS MEDIA PATIENTS</b>
Authors:	Suryani, Zulmardi, Abdi Dharma, Nasril Nasir
Date of receiving by Reviewer:	October 28, 2017
Date of submission From Reviewer:	November 10, 2017

### SECTION II: Comments per Section of Manuscript

General comment:	<b>Good manuscript, but must have repair cause not according to the guidelines, exp. Text layout, tables rules, spacing, references. In abstract, keywords and introduction there is a discussion about vco, but not used in research, in method, result, discussion and conclusion. The aim of this research not according to experiment, result, discussion and conclusion</b>
Introduction:	<b>the relevance of VCO and LAB in this study is not well explained</b>
Methodology:	<b>should be described specific procedures for isolation of pathogenic bacteria. whether isolation using blood so it can be ascertained that grow only pathogenic bacteria only? it is not clear whether the patients were treated with VCO not explained also there are differences in biochemical tests for gram-positive and gram-negative bacteria</b>
Results:	<b>Table not proportional and spacing not consistent The description of the terms and abbreviations of the table does not exist Preferably the percentage of each isolate found in the patient is shown in graphical form</b>



# RASĀYAN Journal of Chemistry

[An International Journal of Chemical Sciences]

(An SCOPUS Indexed Journal, Since 2009)

ISSN: 0974-1496 (Print); ISSN: 0976-0083(Online)

Discussion:	discussion should be improved and adjusted with results, why it is was a few differences with literature
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## SECTION II (Cont.)

Bibliography/References:	the writing of bibliography does not match the guidelines
Others:	
Decision:	

## SECTION III - Please rate the following: (1 = Excellent) (2 = Good) (3 = Fair) (4 = poor)

Originality:	2
Contribution to the Field:	1
Technical Quality:	2
Clarity of Presentation :	3
Depth of Research:	2

## SECTION IV - Recommendation: (Kindly Mark with an X)

Accept As Is:	
Requires Minor Revision:	
Requires Moderate Revision:	X
Requires Major Revision:	
Rejected for publication in RJC(Please give reason):	



# **RASĀYAN** Journal of Chemistry

*[An International Journal of Chemical Sciences]*

**(An SCOPUS Indexed Journal, Since 2009)**

ISSN: 0974-1496 (Print); ISSN: 0976-0083(Online)

## **SECTION V: Additional Comments**

Please add any additional comments (Including comments/suggestions regarding online supplementary materials, if any).



## ANSWERS TO REVIEWER'S COMMENTS'

Reviewer	Comments	Revision Made
1	<p><b>General comment:</b> The work is original and well written, somehow the use of proper English should be implemented in the revision version.</p>	<p>The revision has been made on the use of proper English, proved by the Turnitin checked for the plagiarism and the grammar.</p>
	<p>Introduction: Good to explain the need of the work to be done.</p>	<p>The need of this research has been explained in the Introduction section, in the last paragraph, the third line from the bottom.</p> <p>The most pathogenic bacteria found in CSOM that highlighted CSOM object were <i>Streptococcus Pnemonea</i> and a virus<sup>1</sup>. those pathogenic bacteria previously mentioned were aerobic and anaerobic. <i>P.aeruginosa</i>, <i>S.aureus</i>, <i>S. pyogenes</i>, <i>K.pneumoniae</i>, <i>H.influenzae</i>, <i>Bacteroides</i> and <i>Proteus sp</i> were mostly found along with the mixture of aerobic and anaerobic bacteria that form a layer called biofilm<sup>9</sup>. Meanwhile<sup>10, 11</sup> that there were bacteriocins in Lactid Acid Bacteria (LAB). Bacteriocins can kill pathogenic bacteria but it is not dangerous for non-pathogenic bacteria<sup>12</sup>. Antibacterial test and anti-fungal test using 5 samples of bacteria (<i>E.coli NBRC14237</i>, <i>Staphylococcus aereus NBRC 13276</i>, <i>Bacillus substilis BTCCB</i>, <i>Salmonella thypii</i>, and <i>Listeria monocytogenes</i>) and 2 samples of fungi (<i>Aspergillus niger</i> and <i>Candida sp</i>) in VCO fermentation process, recently found also spices that have the ability as antimicrobial<sup>13</sup>, there were pathogenic bacteria of CSOM patients found among samples of bacteria (<i>S. Aureus</i>). There was a fungus of CSOM patient found between the samples<sup>14</sup>. <b>Because oil layer in VCO is contained LAB that can inhibit the growth of pathogenic bacteria, thus it is hoped that pathogenic bacteria in secretion of CSOM patients can be inhibited as well by the LAB</b></p>
	<p>Methodology:</p>	<p>Thank you for the comment made. Indeed, the methodology has implemented the</p>

	The methodology is the standard one.	standard one.																																											
	<p>Results:</p> <ol style="list-style-type: none"> <li>The results section is detailed and well presented.</li> <li>The tables are needed to be organized to simplify them.</li> </ol>	<ol style="list-style-type: none"> <li>Thank you for the comment on the result section.</li> <li>Below is the revision for the table organization</li> </ol> <p>Previously (before revision)</p> <p style="text-align: center;">Table 1. Sample Distribution</p> <table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th>No.</th> <th>Patient</th> <th>Amount</th> <th>%</th> </tr> </thead> <tbody> <tr> <td>1.</td> <td>Children (under 13 year-old)</td> <td>51</td> <td>40</td> </tr> <tr> <td>2.</td> <td>Adult</td> <td>75</td> <td>60</td> </tr> <tr> <td>3.</td> <td>Male</td> <td>72</td> <td>57</td> </tr> <tr> <td>4.</td> <td>Female</td> <td>54</td> <td>43</td> </tr> </tbody> </table> <p>Revised version</p> <p style="text-align: center;">Table 1. Sample Distribution</p> <table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th>No.</th> <th>Patient</th> <th>%</th> </tr> </thead> <tbody> <tr> <td>1.</td> <td>Children (under 13 year-old)</td> <td>40</td> </tr> <tr> <td>2.</td> <td>Adult</td> <td>60</td> </tr> <tr> <td>3.</td> <td>Male</td> <td>57</td> </tr> <tr> <td>4.</td> <td>Female</td> <td>43</td> </tr> </tbody> </table> <p>Previously (before revision)</p> <p style="text-align: center;">Table 2. Morphologic Analysis of the Isolates</p> <table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th>No.</th> <th>Macroscopic Characteristics of Isolate</th> <th>Isolate</th> <th>Number of Isolate</th> </tr> </thead> <tbody> <tr> <td>1.</td> <td> <ul style="list-style-type: none"> <li>• The color is grayish white</li> <li>• The shape like a fragment</li> </ul> </td> <td><i>Pseudomonas</i></td> <td>74 (58,7%)</td> </tr> </tbody> </table>	No.	Patient	Amount	%	1.	Children (under 13 year-old)	51	40	2.	Adult	75	60	3.	Male	72	57	4.	Female	54	43	No.	Patient	%	1.	Children (under 13 year-old)	40	2.	Adult	60	3.	Male	57	4.	Female	43	No.	Macroscopic Characteristics of Isolate	Isolate	Number of Isolate	1.	<ul style="list-style-type: none"> <li>• The color is grayish white</li> <li>• The shape like a fragment</li> </ul>	<i>Pseudomonas</i>	74 (58,7%)
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			<ul style="list-style-type: none"> <li>• The size is 6-15 mm</li> <li>• The texture is rough</li> <li>• Greenish pigment</li> <li>• Smelly</li> <li>• Gram-negative (bacilli)</li> </ul>	<i>aureginosa</i>	
		2.	<ul style="list-style-type: none"> <li>• Circular shape</li> <li>• The size is medium</li> <li>• Convex</li> <li>• Possessing flagella</li> <li>• Spread</li> <li>• Smell salty</li> <li>• Gram-negative (bacilli)</li> </ul>	<i>Proteus mirabilis</i>	21 (16,6%)
		3.	<ul style="list-style-type: none"> <li>• Circular shape</li> <li>• The size is big</li> <li>• Convex</li> <li>• Muroid</li> <li>• Shiny</li> <li>• The edge is smooth</li> <li>• Gram-negative bacilli</li> </ul>	<i>Klebsiella</i>	7 (5%)
		4.	<ul style="list-style-type: none"> <li>• Circular shape</li> <li>• Slightly Convex</li> <li>• The edge is smooth</li> <li>• The color is yellowish white</li> <li>• The size is 2-5 mm</li> <li>• β hemolytic</li> <li>• Positive-gram (cocci)</li> <li>• Aciniform (Grouped like grapes)</li> </ul>	<i>Staphylococsaureu s</i>	14 (11%)
		5.	<ul style="list-style-type: none"> <li>• Circular shape</li> <li>• Slightly Convex</li> <li>• The edge is smooth</li> <li>• The color is white</li> <li>• The size is small</li> <li>• Cocci</li> <li>• Positive-gram</li> <li>• Aciniform (Grouped like grapes)</li> </ul>	<i>Staphylococcus epidermidis</i>	4 (3%)



Revised version

Table 2. Morphologic Analysis of the Isolates

No.	Macroscopic Characteristics of Isolate	Number of Isolate
1.	<i>Pseudomonas aureginosa</i> <ul style="list-style-type: none"><li>• The color is grayish white</li><li>• The shape like a fragment</li><li>• The size is 6-15 mm</li><li>• The texture is rough</li><li>• Greenish pigment</li><li>• Smelly</li><li>• Gram-negative (bacilli)</li></ul>	(58,7%)
2.	<i>Proteus mirabilis</i> <ul style="list-style-type: none"><li>• Circular shape</li><li>• The size is medium</li><li>• Convex</li><li>• Possessing flagella</li><li>• Spread</li><li>• Smell salty</li><li>• Gram-negative (bacilli)</li></ul>	(16,6%)
3.	<i>Klebsiella</i> <ul style="list-style-type: none"><li>• Circular shape</li><li>• The size is big</li><li>• Convex</li><li>• Mucoid</li><li>• Shiny</li><li>• The edge is smooth</li><li>• Gram-negative bacilli</li></ul>	(5%)
4.	<i>Staphylococcsaureus</i> <ul style="list-style-type: none"><li>• Circular shape</li><li>• Slightly Convex</li><li>• The edge is smooth</li><li>• The color is yellowish white</li><li>• The size is 2-5 mm</li><li>• <math>\beta</math> hemolytic</li><li>• Positive-gram (cocci)</li><li>• Aciniform (Grouped like</li></ul>	(11%)

	grapes)	
5.	<p><i>Staphylococcus epidermidis</i></p> <ul style="list-style-type: none"> <li>• Circular shape</li> <li>• Slightly Convex</li> <li>• The edge is smooth</li> <li>• The color is white</li> <li>• The size is small</li> <li>• Cocci</li> <li>• Positive-gram</li> <li>• Aciniform (Grouped like grapes)</li> </ul>	(3%)

Previously (before revision)

**Table 3. Morphologic Analysis of Fungi from the isolates of Pathogenic Bacteria**

No.	Characteristics	Isolate	No of Isolate
1.	<ul style="list-style-type: none"> <li>• Positive-gram</li> <li>• Pseudohypha +</li> <li>• Didn't grow in blood agar</li> <li>• Grow in saboraaud</li> <li>• Circular shape, white, and slightly mucoid</li> </ul>	<i>Candida sp</i>	6 (4,7%)

Revised version

**Table 3. Fungi Morphologic Analysis from Pathogenic Bacteria isolates**

No.	Characteristics	No of Isolate
1.	<i>Candida sp</i> <ul style="list-style-type: none"><li>• Positive-gram</li><li>• Pseudohypha +</li><li>• Absent growth in blood agar</li><li>• Present in saboraud media</li><li>• Circular in shape, white in color, and slightly mucoid</li></ul>	(4,7%)

Previously (before revision)

**Table 4. Result of Biochemical Test of the Isolate**

No.	Test	Result	No of Isolate	Isolate
1.	TSIA	K/K	74	<i>Pseudomonas aureginosa</i>
2.	Gas	+		
3.	H <sub>2</sub> S	-		

			4.	SC	+		
			5.	Sulfur	+		
			6.	Indole	-		
			7.	Motile	+		
			1.	TSIA	K/A	21	<i>Proteus mirabilis</i>
			2.	Gas	+		
			3.	H <sub>2</sub> S	+		
			4.	SC	+		
			5.	Sulfur	+		
			6.	Indole	-		
			7.	Motile	+		
			1.	TSIA	A/A		<i>Klebsiella</i>
			2.	Gas	+		
			3.	H <sub>2</sub> S	-		
			4.	SC	+		

			5.	Sulfur	-		
			6.	Indole	-		
			7.	Motile	-		
			1.	Catalase	+	14	<i>Staphylococcus aureus</i>
			2.	Gas	+		
			3.	Coagulase	+		
			4.	Novobio cin	Sensiti ve		
			1.	Catalase	+	4	<i>Staphylococcus epidermidis</i>
			2.	Gas	+		
			3.	Coagulase	-		
			4.	Novobio cin	Sensiti ve		

Revision version

Table 4. Result of Biochemical Test of the Isolate

		<table border="1"> <thead> <tr> <th rowspan="2">Isolat</th> <th colspan="7">T e s t</th> </tr> <tr> <th>Catalase</th> <th>Ga s</th> <th>Coa la gu se</th> <th>Nov biocin o</th> <th colspan="3"></th> </tr> </thead> <tbody> <tr> <td><i>Staphylococcus epidermidis</i></td> <td>+</td> <td>+</td> <td>-</td> <td></td> <td></td> <td colspan="2">sensit ive</td> </tr> <tr> <td><i>Staphylococcus aureus</i></td> <td>+</td> <td>+</td> <td>+</td> <td></td> <td></td> <td colspan="2">sensit ive</td> </tr> <tr> <td></td> <th colspan="7">T e s t</th> </tr> <tr> <td></td> <th>TSIA</th> <th>Ga s</th> <th>H2S</th> <th>S C</th> <th>Sulf ur</th> <th>Indol e</th> <th>Moti le</th> </tr> <tr> <td><i>Klebsiella</i></td> <td>A/A</td> <td>+</td> <td>-</td> <td>+</td> <td>-</td> <td>-</td> <td>-</td> </tr> <tr> <td><i>Proteus mirabilis</i></td> <td>K/A</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td>-</td> <td>+</td> </tr> <tr> <td><i>Pseudomonas aureginosa</i></td> <td>K/K</td> <td>+</td> <td>-</td> <td>+</td> <td>+</td> <td>-</td> <td>+</td> </tr> </tbody> </table>	Isolat	T e s t							Catalase	Ga s	Coa la gu se	Nov biocin o				<i>Staphylococcus epidermidis</i>	+	+	-			sensit ive		<i>Staphylococcus aureus</i>	+	+	+			sensit ive			T e s t								TSIA	Ga s	H2S	S C	Sulf ur	Indol e	Moti le	<i>Klebsiella</i>	A/A	+	-	+	-	-	-	<i>Proteus mirabilis</i>	K/A	+	+	+	+	-	+	<i>Pseudomonas aureginosa</i>	K/K	+	-	+	+	-	+
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	<p>Discussion: It is still needed to discuss the results further, compare them with existing data to explain the findings.</p>	<p>Thank you for the comment, and the revision has been made in the last paragraph. The additional discussion was started from the 4<sup>th</sup> line from the bottom of the last paragraph. It was highlighted in blue color.</p> <p>Additional version: The result of this research was also similar to the one conducted by another expert <sup>19</sup>, consisting of <i>Pseudomonas aureginosa</i> 40%, <i>Proteus</i> 5%, <i>Klebsiella</i> 5%, and <i>Staphylococcus</i> 31%. The difference was, there were two types of the <i>Staphylococcus</i> found; <i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i>. <i>Eschericia coly</i> 12% was found in <sup>19</sup>, in contrast with this research where none found.</p>																																																																							
	<p>Bibliography/References: The references used in this article are the updated ones.</p>	<p>Thank you for the comment on the references, and actually there are 3 new references taken from Rayasan have been referred to.</p>																																																																							
	<p>Others:</p>	<p>Simplification on the Acknowledgment section.</p>																																																																							



	<p>Aknowledgment section should be simplified.</p>	<p>Previously (before revision)</p> <p style="text-align: center;"><b>ACKNOWLEDGEMENT</b></p> <p>This research would never be finished without helps form other people, and we want to thank everybody that helped us to finish this research:</p> <ol style="list-style-type: none"> <li>1. Director of DRPM of Ministry of Research, Technology and Higher Education of the Republic of Indonesiafor funding this research throughFirst-year Fundamental Donation, contract number:</li> <li>2. Prof.Dr. Rahmiana Zein that helped us from the beginning of this research.</li> <li>3. The Head of Dr. M. Djamil Hospital that has given us permission to do this research.</li> <li>4. The Head of Basic Laboratory of Kopertis X that also gave us permission and help to finish this research.</li> </ol> <p>Revised Version</p> <p style="text-align: center;"><b>ACKNOWLEDGEMENT</b></p> <p>This research would never be finished without helps form other people, and we want to thank everybody that helped us to finish this research:</p> <ol style="list-style-type: none"> <li>1. Director of DRPM of Ministry of Research, Technology and Higher Education of the Republic of Indonesia for the First-Year Fundamental Donation grant given.</li> <li>2. Prof. Dr. Rahmiana Zein; Post Graduate lecturer.</li> <li>3. The Head of Dr. M. Djamil General Hospital for the hospital facility used.</li> </ol>
2.	<p>General comment: Overall, this research has implemented the right and good</p>	<p>Thank you for the comment made. The method has been explained. None to be revised</p>

	<p>methods in order to get rich data for its result. The methods which are conducted here are Isolation of pathogenic bacteria of 126 CSOM patients, and Identification of pathogenic bacteria using gram-negative and positive test, as have been proposed by experts in their researches.</p>	
	<p><b>Introduction:</b> This introduction has fulfilled the requirement of what should be stated as the background of the research. Rich information of the topic of this research and provide it with experts statement as well that concerns on the similar field.</p>	<p>Thank you for the comment made. The Introduction has fulfilled the criteria of this journal, as it has been explained concisely. None to be revised</p>
	<p><b>Methodology:</b> It has applied the appropriate methods which should be done within the research analyzing Pathogenic Bacteria on the secretion of chronic suppurative otitis media patients. It can be seen from the rich data obtained.</p>	<p>Thank you for the comment made. The methodology has been explained in accordance with the criteria. None to be revised</p>
	<p><b>Results:</b> The result gives depth analysis on the data perceived by providing it with particular table for particular</p>	<p>Thank you for the comment made. The result has been discussed and fulfilled the criteria. None to be revised</p>

	focus of concern.	
	<p>Discussion: The discussion shows its clarity in presentation by elaborating the data within each table concisely and neatly.</p>	<p>Thank you for the comment made. The result has been explained clearly. None to be revised</p>
	<p>Bibliography/References: The references taken as the guideline for conducting this research are appropriate and fit with the topic of discussion.</p>	<p>Thank you for the comment made. The bibliography has been written referred to the template given. And eliminated the articles in Bahasa Indonesia.</p> <p style="text-align: center;"><b>REFERENCES</b></p> <ol style="list-style-type: none"> <li>1. Moorthy, P. N. S., Lingaiah, J., Katari, S. &amp; Nakirakanti, A. Clinical Application of a Microbiological Study on Chronic Suppurative Otitis Media. <i>Int. J. Otolaryngology Head Neck Surg.</i> <b>2013</b>, 290–294 (2013).</li> <li>2. Massa, H. M., Cripps, A. W., Lehmann, D. &amp; Journal, M. Otitis media: viruses, bacteria, biofilms and vaccines. <i>MJA</i> <b>191</b>, 4–9 (2009).</li> <li>3. Prakash Adikari, S. Chronic Suppurative Otitis Media in urban private school children of Nepal. <i>Braz. J. Otorhinolaryngol</i> <b>75</b>, 2007–2010 (2009).</li> <li>4. Pradesh, A. Aerobic bacteriology of chronic suppurative otitis media in Rajahmundry , Andhra Pradesh , India. <b>4</b>, 73–79 (2012).</li> <li>5. Asroel, H. A., Siregar, D. R. &amp; Aboet, A. Profil of Patient with Chronic Suppurative Otitis Media. <i>J. Kesehatan. Masy. Nas.</i> <b>7</b>, 567–571 (2010).</li> <li>6. Rinny Olivia Sembiring, Jon Porotu'o, O. waworuntu. ANTIBIOTIK PADA PENDERITA TONSILITIS DI POLIKLINIK THT-KL BLU RSU . PROF . DR .</li> </ol>

		<p>R . D . KANDOU MANADO. <i>J. e-Biomedik</i><b>1</b>, 1053–1057 (2013).</p> <p>7. Sembiring, R. O. &amp; Waworuntu, O. IDENTIFIKASI BAKTERI UJI KEPEKAAN TERHADAP ANTIBIOTIK PADA PENDERITA TONSILITIS DI POLIKLINIK THT-KL BLU RSU . PROF . DR . R . D . KANDOU MANADO. <i>J. e-Biomedik</i><b>1</b>, 1053–1057 (2013).</p> <p>8. Hilma Kholida. <i>Biofilm Formation in clinical isolate of pseudomonas sp causes of Chronic suppurative Otitis Media</i>. 177 (2012).</p> <p>9. Homenta, H. Infeksi biofilm bakterial. <i>Homenta</i><b>4</b>, 1–11 (2016).</p> <p>10. Suryani, Dharma, A., Manjang, Y., Arief, S., Munaf, E. &amp; Nasir, N. Antimicrobial and Antifungal Activity of Lactic Acid Bacteria Isolated from Coconut Milk Fermentation . <i>Res. J. Pharm. Biol. Chem. Sci.</i><b>5</b>, 1587–1595 (2014).</p> <p>11. Bl, S., Shrestha, I. &amp; Rc, A. Comparison of clinical presentation between Chronic Otitis Media Mucosal with Squamous . <i>Orig. Artic.</i><b>8</b>, 387–391 (2010).</p> <p>12. Nguyen, H. T. H., Elegado, F. B., Mabesa, R. C. &amp; Dizon, E. I. Isolation and characterisation of selected lactic acid bacteria for improved processing of Nem chua , a traditional fermented meat from Vietnam. <i>Benef. Microbes</i><b>1</b>, 67–74 (2010).</p> <p>13. Hamad, A., Mahardika, M. G. P., Yuliani, I. &amp; Hartanti, D. CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OILS OF <i>Syzygium polyanthum</i> AND <i>Syzygium aromaticum</i>. <i>Rasayan J.Chem</i><b>10</b>, 564–569 (2017).</p> <p>14. Suryani, A. D. Isolation and Characterization of Bacteriocins Bacteria <i>Lactobacillus Plantarum</i> Strain NM178-5 from Fermentation Process with</p>
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		<p>Contained on Coconut Milk. <i>Transylvanian Reviwer</i> <b>XXIV</b>, 614–628 (2016).</p> <p>15. Yaor, M. A. &amp; Jafari, B. Surgical Management of Chronic Suppurative Otitis Media : A 3-year Experience. <i>Annals African Med.</i> <b>5</b>, 24–27 (2006).</p> <p>16. R Shyamala, Ps. The study of bacteriological agents of chronic suppurative otitis media - Aerobic culture and evaluation. <i>J. Microbiol. Biotechnol. Res.</i> <b>2</b>, 152–162 (2012).</p> <p>17. Prakash, M., Lakshmi, K., Anuradha, S. &amp; Gn, S. BACTERIOLOGICAL PROFILE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN OF CASES OF CHRONIC SUPPURATIVE OTITIS MEDIA. <i>Asian J. Pharm. Clin. Res.</i> <b>6</b>, 5–7 (2013).</p> <p>18. Suryani, Dharma, A., Manjang, Y., Muhammadiyah, U., Barat, S. &amp; Andalus, U. ISOLASI BAKTERI PATOGEN PADA PASIEN PENDERITA INFEKSI TELINGA Chronic supparative otitis media ( OMSK ). <i>KATALISATOR</i> 1–10 (2016).</p>
	<p>Others: Perhaps, it needs a bit addition on</p>	<p>Thank you for the comment made. The discussion regarding to the result on the paragraph below the Table 1 has been</p>

the conclusion part. Develop the result paragraph by presenting more brief explanation about them in order to make the data becoming more credible.

compared with researches conducted by other experts like Yaor MA and Shyamala. The discussion made was shown below:

The table above showed that 57% of the data were taken from male. The patients chosen were 40% children and 60% adult. Yaor, MA in his previous research said that CSOM can attack children and adult<sup>15</sup>. He confirmed that of 73 studied CSOM patients aged 9 to 84 year-old, 17 of them (24%) were children aged 9-15. The 40% number occurred to children were because of poor hygiene practice therefore was easily infected by the bacteria and another side Shyamala found that 70% of CSOM patients were children aged 0-20. It was similar with Moris statement that mostly those who suffer CSOM were children.<sup>16, 4, 3, 17</sup>

The references for the discussion of Table 2, 3, and 4 have been made, as seen below:

From morphology identification result mentioned in Table 2 and 3, it can be seen the shape, color, size of colony from each isolate and also the gram test result. The above result showed the types of pathogenic bacteria in the secretion of CSOM patients in X Hospital, they were *Pseudomonas aureginosa* (58,7%), *Staphilococcus aureus* (11%), *Staphilococcus epidermidis* (3%), *Proteus mirabilis* (16,6%), *Klebsiela sp* (5%) and 1 fungi *Candida sp* (4,7%). This result is supported by the result of biochemical test on each isolate such as Catalase, Coagulase, formed gas, and Novobiocin test as stated in table 4. Nevertheless the result was in the same agreement with other experts, there was a few differences on the pathogenic bacteria and fungi found in the secretion of CSOM patients. Sthrestha et.al (2011) said that pathogenic bacteria and pathogenic fungus of CSOM patients were *Staphylococcus aureus* 32,2%, *Streptococcus pnemoni* 6,1%, *Pseudomonas aureginosa* 26,9%, *Klebsiella sp* 10,4%, *Proteus mirabilis* 6,9%, *E.coli* 6,9%, fungi *Aspergillus sp* 6,9% *Candida sp* 2,6%.

Additional version:

Sthrestha et.al (2011) said that pathogenic bacteria and pathogenic fungus of CSOM

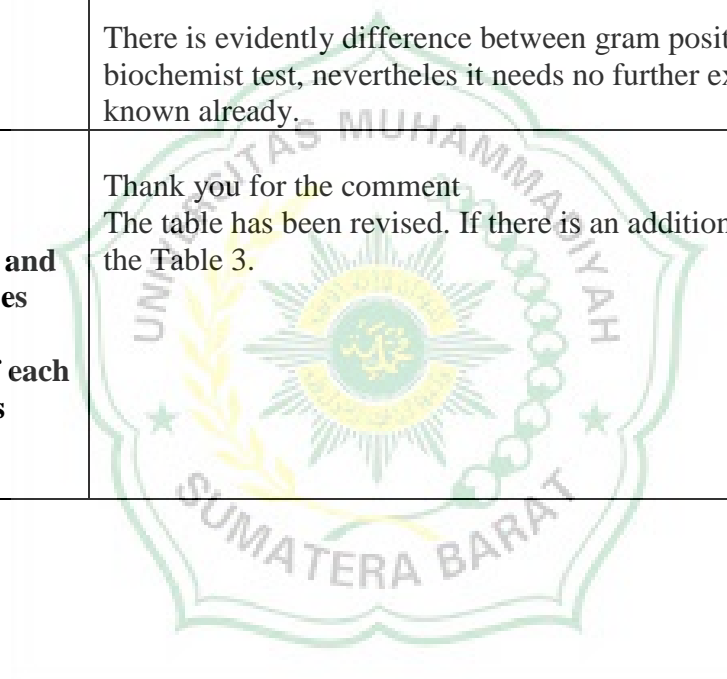
		<p>patients were <i>Staphylococcus aureus</i> 32,2%, <i>Streptococcus pnemoni</i> 6,1 %, <i>Pseudomonas aureginosa</i> 26,9 % , <i>Klebsiella sp</i> 10,4 % , <i>Proteus mirabilis</i> 6,9 % , <i>E.coli</i> 6,9%, fungi <i>Aspergillus sp</i> 6,9 % <i>Candida sp</i> 2,6 % . The result of this research was also similar to the one conducted by another expert <sup>19</sup> , consisting of <i>Pseudomonas aureginosa</i> 40%, <i>Proteus</i> 5%, <i>Klebsiella</i> 5%, and <i>Staphylococcus</i> 31%. The difference was, there were two types of the <i>Staphylococcus</i> found; <i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i>. <i>Eschericia coly</i> 12% was found in<sup>19</sup> , in contrast with this research where none found.</p> <p>There was additional discussion for the conclusion part as shown below:</p> <p style="text-align: center;"><b>CONCLUSION</b></p> <p>From the result mentioned above, it can be concluded that there were 126 isolates of pathogenic bacteria from the secretion of 96 CSOM patients. More over, there were 5 kinds of pathogenic bacteria found in the secretion of CSOM patients in X Hospital; <i>Pseudomonas aeruginosa</i>; <i>Klebsiella</i>, <i>Proteus</i>; <i>Staphylococcus aureus</i>; <i>Staphilococcus epidermidis</i> and one species of fungi <i>Candida spp</i>.</p> <p>Additional version :</p> <p>From the result mentioned above, it can be concluded that there were 126 isolates of pathogenic bacteria from the secretion of 96 CSOM patients. More over, there were 5 kinds of pathogenic bacteria found in the secretion of CSOM patients in X Hospital; <i>Pseudomonas aeruginosa</i> ( coloni/ % ); <i>Klebsiella</i> ( coloni/ % );, <i>Proteus</i> ( coloni/ % ) ; <i>Staphylococcus aureus</i> ( coloni/ % );; <i>Staphilococcus epidermidis</i> ( coloni/ % ); and one species of fungi <i>Candida spp</i>.</p>
3.	<p>General comment: Good manuscript, but must have repair cause not according to the guidelines, exp. Text layout, tables rules, spacing, references.</p>	<p>Thank you for the comment made. The writing on this manuscript has been in accordance with the template given.</p>

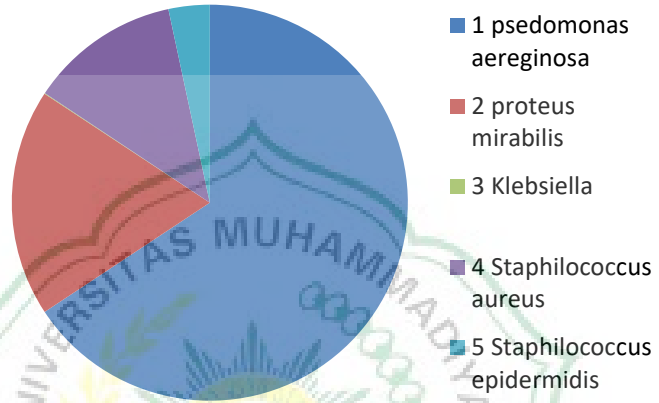


	<p>In abstract, keywords and introduction there is a discussion about vco, but not used in research, in method, result, discussion and conclusion. The aim of this research not according to experiment, result, discussion and conclusion</p>	
	<p>Introduction:  <b>The relevance of VCO and LAB in this study is not well explained</b></p>	<p>The relevance of VCO and LAB within this research has been clearly explained in the last paragraph of Introduction section, they are:</p> <p>Because oil layer in VCO is contained LAB that can inhibit the growth of pathogenic bacteria, thus it is hoped that pathogenic bacteria in secretion of CSOM patients can be inhibited as well by the LAB</p>
	<p>Methodology:  <b>Should be described specific procedures for isolation of pathogenic bacteria, whether isolation using blood so it can be ascertained that grow only pathogenic bacteria only? it is not clear whether the patients were treated with VCO not explained also there are differences in biochemical tests for gram-positive and gram-negative bacteria</b></p>	<p>Thank you for the comment made.</p> <p>To be more precise, in General Procedure on the 5<sup>th</sup> line after Mc Conkey word should be more additional information, so the sentences would be like shown below:</p> <p>This media is a selective media for pathogenic bacteria. Non pathogenic bacteria cannot grow in this media.</p> <p><b>General procedure</b>  <i>The Isolation of Pathogenic Bacteria in the Secretion of the Patients.</i> The isolation stage was done before doing the identification of pathogenic bacteria in the secretion of 126 CSOM patients. Pathogenic bacteria from 126 CSOM patients were isolated by using dilution method up to 10<sup>-7</sup> dilution level, whereas the media used to isolate these bacteria were blood agar and McConkey agar.</p> <p>It has been clearly explained that patients were not treated by VCO. The sample was directly taken from the CSOM patient secretion.</p>



		<p>It can be seen from Methodology section, on the 1<sup>st</sup> line:</p> <p><b>Methods</b>  There were 2 stages conducted in this study; (1) Isolation of pathogenic bacteria of 126 CSOM patients;</p> <p>There is evidently difference between gram positive and gram negative bacteria on the biochemist test, nevertheles it needs no further explanation as this information should be known already.</p>
	<p>Results:  <b>Table not proportional and spacing not consistent</b>  <b>The description of the terms and abbreviations of the table does not exist</b>  <b>Preferably the percentage of each isolate found in the patient is shown in graphical form</b></p>	<p>Thank you for the comment  The table has been revised. If there is an additional graphic, so it would be written below the Table 3.</p>

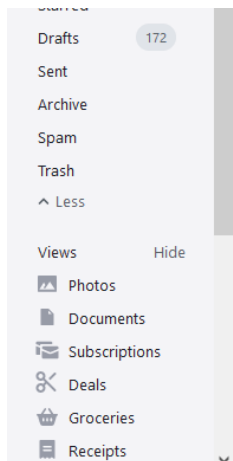


		<p style="text-align: center;">%</p>  <p>1 Pseudomonas aeruginosa 2 Proteus mirabilis 3 Klebsiella 4 Staphylococcus aureus 5 Staphylococcus epidermidis</p>	
	<p>Discussion: <b>Discussion should be improved and adjusted with results, why it is was a few differences with literature</b></p>	<p>Thank you for the comment. On the last line of the last paragraph in the Discussion section, there is an additional: The difference on the result of this pathogenic microba occurred due to the environment or different area, so the microba of pathogenic bacteria grown in the patient secretion is different as well.</p>	
	<p>Bibliography/References: <b>The writing of bibliography does not match the guidelines</b></p>	<p>The writing of bibliography has matched with the template given, and the sample of the articles published in Rayasan Journal.</p> <p>Taken as the example on the 1<sup>st</sup> paragraph, the 2<sup>nd</sup>, the 3<sup>rd</sup>, the 6<sup>th</sup>, and the 8<sup>th</sup> line in the Introduction section, the writing of the bibliography uses number written on the upper</p>	

		<p>right. Similar with the sample, where the writing for the bibliography uses number written on the upper right</p> <p>Furthermore, the bibliography writing has been utilized Mendeley Application, therefore it can minimize the error in writing the bibliography.</p>
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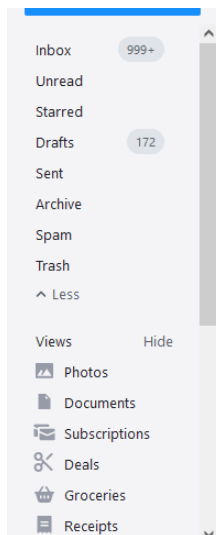
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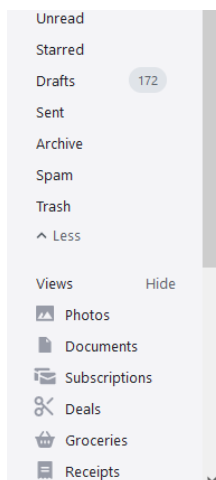
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First of all on behalf of other authors, I would like an apology for being late replying your email and sending the revision made on our manuscript. It was because your last email sent cannot be accessed, thus I do not know the information of publishing the manuscript in April. The email that we have been contacted each other before has been through maintenance process. As a result, I use this email as an alternate email in contacting you. Regarding to this matter, I hope that this manuscript can be published in July, 2018. And I also would like to inform you, that I have paid for the journal fee.

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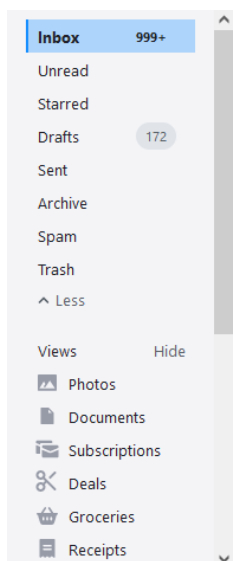
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# ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA SECRETION OF CHRONIC SUPPURATIVE OTITIS MEDIA PATIENTS

**Suryani Suryani<sup>1,\*</sup>, Zulmardi<sup>2</sup>, Abdi Dharma<sup>3</sup> and Nasril Nasir<sup>4</sup>**

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## ABSTRACT

The aims of this research were to isolate and identify the pathogenic bacteria in the secretion of Chronic Suppurative Otitis Media (CSOM) patients as the development of Lactic Acid Bacteria (LAB) analysis in Virgin Coconut Oil (VCO) fermentation process. It is expected that LAB in the VCO could be antimicrobial/antibacterial of bacteria in the secretion of CSOM patients. There were 2 stages conducted within this research, firstly using blood agar and dilution method in isolating the bacteria in CSOM patients's secretion. Secondly, the isolates were identified morphologically, physiology, and another biochemical test. There are 126 isolates and 5 kinds of pathogenic bacteria (*Pseudomonas aureginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Klebsiella Sp*) and one kind of fungi (*Candida sp*) as the result. The samples of CSOM patients are 60% above aged 20 and 40% below it, and an equal balance of percentage between male and female.

**Keywords:** Pathogenic bacteria isolation, Secretion of CSOM patients, Chronic Suppurative Otitis Media, Virgin Coconut Oil (VCO), Lactic Acid Bacteria (LAB).

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## INTRODUCTION

Chronic Suppurative Otitis Media (CSOM) is one of the ear diseases that commonly suffered by children and causes deafness, even death<sup>1,2</sup>. It usually attacks people in developing countries such as India, Nepal, Vietnam and also Indonesia<sup>3,4</sup>. Indonesian calls it 'congek', and is one of the deadly diseases because there are tympanic membrane perforation and secretion that flows from the outer ear continuously or temporary which can cause dangerous complication such as brain abscess and meningitis<sup>5</sup>. CSOM derives from the late effect of treatment for acute otitis media patient, or poor hygiene practice, high virulence, and a weak immune system due to malnutrition<sup>6</sup>.

Some researchers have tried to isolate the pathogenic bacteria in the secretion of CSOM patients, and one of them was an Indian researcher<sup>7</sup>. He said that from 80 samples of CSOM patients, there were few pathogenic bacteria; *Staphylococcus aureus*, *Pseudomonas sp*, *Escherichia coli*, and *Klebsiella sp*. Apparently, 18% of the bacteria were resistance toward antibiotic like methicillin, and sensitive toward amikacin, chloramfenicol and piperacillin.

The most pathogenic bacteria found in CSOM that highlighted CSOM object were *Streptococcus Pnemonea* and a virus<sup>1</sup>. Those pathogenic bacteria previously mentioned were aerobic and anaerobic. *P.aeruginosa*, *S.aureus*, *S. pyogenes*, *K.pneumoniae*, *H.influenzae*, *Bacteroides* and *Proteus sp* were mostly found along with the mixture of aerobic and anaerobic bacteria that form a layer called biofilm<sup>8</sup>.

Meanwhile<sup>9</sup> that there were bacteriocins in Lactic Acid Bacteria (LAB). Bacteriocins can kill pathogenic bacteria but it is safe for non-pathogenic bacteria<sup>10, 11, 12</sup>. Antibacterial test and antifungal test using 5 samples of bacteria (*E.coli* NBRC14237, *Staphylococcus aureus* NBRC 13276, *Bacillus subtilis* BTCCB, *Salmonella thypii*, and *Listeria monocytogenes*) and 2 samples of fungi (*Aspergillus niger* and *Candida sp*) in VCO fermentation process, recently found also spices that have the ability as antimicrobial<sup>9,13,14,15</sup> there were pathogenic bacteria of CSOM patients found among samples of bacteria (*S. Aureus*). There was a fungus of CSOM patient found between the samples<sup>11</sup>.



Because the oil layer in VCO is contained LAB that can inhibit the growth of pathogenic bacteria, thus it is hoped that this bacteria in patients secretion can be inhibited as well by the LAB.

## EXPERIMENTAL

### Materials

Material for this study was ear liquid of 126 CSOM patients in RSUP DR. M. Jamil Padang, Indonesia Hospital. Blood and McConkey agar was the media used to grow the bacteria during conventional isolation and identification processes.

### Methods

Previously, 2 stages were implemented before going through the identification process. They were by isolating the pathogenic bacteria taken from 126 CSOM patients. Then identifying them using gram-negative and positive test, bacterial staining test, morphology test, and biochemical test including catalase test and other carbohydrate tests.

### General Procedure

#### Pathogenic Bacteria Isolation in the Patients Secretion

The bacteria identification in 126 CSOM patients secretion was begun with the isolation stage. The dilution method was taken in isolating the bacteria which was up to  $10^{-7}$  dilution level, whilst the media used were blood and McConkey agar. Streaking the bacteria to form it into a single colony was done so it could become the isolate of the pathogenic bacteria. At the same time when the secretion was scratched in blood agar, it was also enriched in tiogikolat. When there was no bacterium grew in the media, then continued to enriching and planting the sample within blood agar media. Usually, in each CSOM patient, there was one isolate produced.

#### The Identification of Pathogenic Bacteria Isolate

Then the collected isolates were morphologically identified referred to their colony pattern and color. Besides that, positive and negative-gram tests, a biochemical tests like catalase test, starch test, and novobiocin test were also performed.

## RESULTS AND DISCUSSION

Seen in the Table-1 below is the data of secretion taken from CSOM patients.

Table-1 Sample Distribution

No.	Patient	%	Patient	%
1.	Children (under 13 year-old)	40	Male	57
2.	Adult	60	Female	43

The table above showed that 57% of the data were taken from the male. The patients chosen were 40% of children and 60% adult. Yaor, MA in his research stated that CSOM can affect children and adult<sup>16,17</sup>. He confirmed that of 73 studied CSOM patients aged 9 to 84 year-old, 17 of them (24%) were children aged 9-15. The 40% number occurred for children were because of poor hygiene practice, therefore, was easily infected by the bacteria and another side Shyamala found that 70% of CSOM patients were children aged 0-20. It was similar with Moris statement that mostly those who suffer CSOM were children<sup>3,18,19</sup>. Accordingly,<sup>20</sup>, were children 0-10 years old 20% .

#### The Isolation of Pathogenic Bacteria

This study found one kind of pathogenic bacteria of the CSOM patient in the isolation process, so there were 126 isolates gathered at the end. Compared to Suryani, and R. Shyamala<sup>19, 21, 21</sup> study, he stated that each patient had one isolate; 64% of 192 samples, while 34% of them had more than one, and 5.33% of the isolated secretion produced fungi. So also with Strestha get mushrooms as much as 10,3% from 230 sample<sup>22</sup>.

### Morphologic Identification of Pathogenic Bacteria

The bacteria identification result of 126 secretions of CSOM patients can be seen in Table-2.

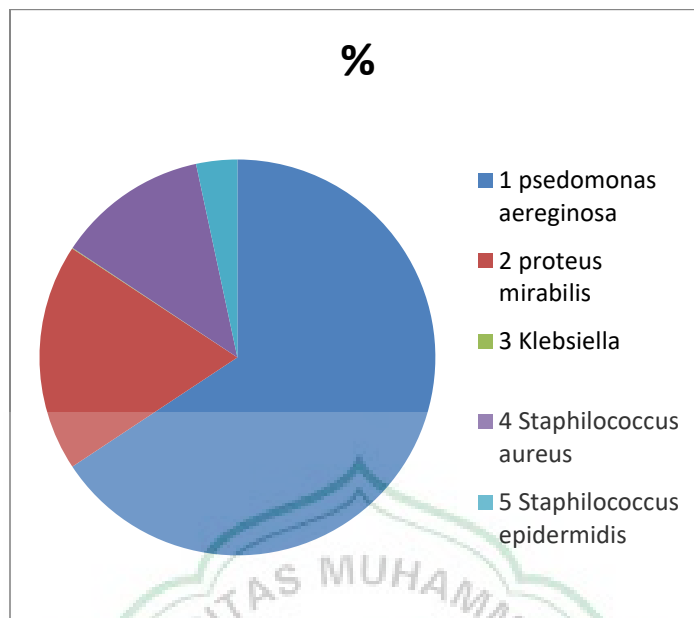


Fig.-1: Percentage of Isolates Types Morphology

Table-2: Isolates Morphologic Analysis

No.	Isolate Macroscopic Characteristics	No of Isolate
1.	<p><i>Pseudomonas aureginosa</i></p> <ul style="list-style-type: none"> <li>• Grayish white in color</li> <li>• Fragment like shape</li> <li>• 6-15 mm in size</li> <li>• Rough in texture</li> <li>• Greenish pigment</li> <li>• Smelly</li> <li>• Gram-negative (bacilli)</li> </ul>	(58,7%)
2.	<p><i>Proteus mirabilis</i></p> <ul style="list-style-type: none"> <li>• Circular in shape</li> <li>• Medium in size</li> <li>• Convex</li> <li>• Possessing flagella</li> <li>• Spread</li> <li>• Smell salty</li> <li>• Gram-negative (bacilli)</li> </ul>	(16,6%)
3.	<p><i>Klebsiella</i></p> <ul style="list-style-type: none"> <li>• Circular in shape</li> <li>• Big in size</li> <li>• Convex</li> <li>• Muroid</li> <li>• Shiny</li> <li>• Smooth edge</li> <li>• Gram-negative bacilli</li> </ul>	(5%)
4.	<p><i>Staphylococcsaureus</i></p> <ul style="list-style-type: none"> <li>• Circular in shape</li> <li>• Slightly Convex</li> <li>• Smooth edge</li> </ul>	(11%)

	<ul style="list-style-type: none"> <li>• Yellowish white in color</li> <li>• 2-5 mm in size</li> <li>• <math>\beta</math> hemolytic</li> <li>• Positive-gram (cocci)</li> <li>• Grouped like grapes (Aciniform)</li> </ul>	
5.	<i>Staphylococcus epidermidis</i> <ul style="list-style-type: none"> <li>• Circular in shape</li> <li>• Slightly Convex</li> <li>• Smooth edge</li> <li>• White in color</li> <li>• Small in size</li> <li>• Cocci</li> <li>• Positive-gram</li> <li>• Grouped like grapes (Aciniform)</li> </ul>	(3%)

For fungi identification, there was a colony found with the presence of hypha during the observation of the isolation process. Then, continued by gram staining test in order to obtain positive pseudohyphae as the result. Further, growing the samples in blood agar and Sabouraud agar. Resulted that the colony grew in Sabouraud agar instead of blood agar, in the form of circular shape, white, and slightly mucoid. The result showed in Table-3 below:

Table-3: Fungi Morphologic Analysis from Pathogenic Bacteria Isolates

No.	Characteristics	No of Isolate
1.	<i>Candida sp</i> <ul style="list-style-type: none"> <li>• Positive-gram</li> <li>• Pseudohypha +</li> <li>• Absent growth in blood agar</li> <li>• Present in sabotaged media</li> <li>• Circular in shape, white in color, and slightly mucoid</li> </ul>	(4,7%)

### Biochemical Test

The results of isolates biochemical test can be viewed in Table-4.

Table-4: Result of Biochemical Test of the Isolates

Isolates	T e s t						
	Catalase	Gas	Coagu	lase	Novo	biocin	
<i>Staphylococcus epidermidis</i>	+	+	-			sensitive	
<i>Staphylococcus aureus</i>	+	+	+			sensitive	
Isolates	T e s t						
	TSIA	Gas	H2S	SC	Sulfur	Indole	Motile
<i>Klebsiella</i>	A/A	+	-	+	-	-	-
<i>Proteus mirabilis</i>	K/A	+	+	+	+	-	+
<i>Pseudomonas aureginosa</i>	K/K	+	-	+	+	-	+

From morphology identification result mentioned in Table-2 and 3, the shape, color, size of the colony from each isolate and also the gram test result are shown. The above result showed the types of pathogenic bacteria in CSOM patients secretion in X Hospital, they were *Pseudomonas aureginosa*(58,7%), *Staphilococcus aureus* (11 %), *Staphilococusepidermidis*(3%), *Proteus mirabilis* (16,6 %), *Klebsiella sp* (5%)and 1 fungi *Candida sp* (4,7%). This result was in accordance with biochemical test result on each isolate such as Catalase, Coagulase, formed a gas, and Novobiocin test as stated in Table 4. Nevertheless the result was in the same agreement with other experts, there were a few differences on the pathogenic bacteria and fungi found in the secretion of CSOM patients.

Sthrestha, 2011<sup>22</sup> said that pathogenic bacteria and pathogenic fungus of CSOM patients were *Staphylococcus aureus* 32,2%, *Streptococcus pneumoniae* 6,1 %, *Pseudomonas aeruginosa* 26,9 % , *Klebsiella sp* 10,4 %, *Proteus mirabilis* 6,9 %, *E.coli* 6,9%, fungi *Aspergillus sp* 6,9 % *Candida sp* 2,6 %. Accordingly<sup>7</sup>, pathogenic bacteria of CSOM patient were *Staphylococcus aureus* 41,25%, *Escherichia coli* 5%, *Klebsiella pneumoniae* 7,5%, *Proteus sp* 5% and *Pseudomonas sp* 37,5%.

### CONCLUSION

From the result mentioned above, it can be concluded that there were 126 isolates of pathogenic bacteria from the secretion of 96 CSOM patients. More over, by applying morphological, physical and biochemical test analysis of identification, there were 5 kinds of pathogenic bacteria found in patients secretion taken from X Hospital; *Pseudomonas aeruginosa*; *Klebsiella*, *Proteus*; *Staphylococcus aureus*; *Staphylococcus epidermidis* and one species of fungi *Candida sp*.

### ACKNOWLEDGEMENT

This research would never be finished without helps from other people, and we want to thank everybody that helped us to finish this research:

1. Director of DRPM of Ministry of Research, Technology and Higher Education of the Republic of Indonesia for the First-Year Fundamental Donation grant given.
2. Prof. Dr. Rahmiana Zein; Post Graduate lecturer.
3. The Head of Dr. M. Djamil General Hospital for the hospital facility used.

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